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AN INSILICO DOCKING STUDY OF *Chromolaena odorata* DERIVED COMPOUNDS AGAINST ANTIMALARIAL ACTIVITY

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ABSTRACT: ***Malaria remains one of the major public health problems when Plasmodium falciparum is one of the causative agents. The dihydrofolate reductase (DHFR) is one of the well-defined targets of P. falciparum which is involved in the reproduction of this parasite. Proguanil is a prophylactic antimalarial drug; it stops the malaria parasite, from reproducing once it is in the red blood cells. It does this by inhibiting the enzyme, dihydrofolate reductase. The side effects of these drugs make the need for the necessity of new improved drugs. The present paper was framed to find active components through GC MS analysis and insilico docking studies with the identified compounds in the methanolic extract of Chromolaena odorata leaves to validate the antimalarial potential of these phytocompounds. There are 33 phytocompounds derived through GC-MS analysis, out of these 4 compounds satisfied the Lipinski's properties. The docking studies of these compounds were done using commercial tool Accelyrs Discovery Studio 2.1. Among these compounds, Falcarinol showed the highest dock score of 71.128 with more hydrogen bond interactions. The results suggested that Falcarinol will act against malaria by blocking the DHFR receptor and also it can be developed into a powerful drug for malaria in future.***

1. INTRODUCTION:

Malaria is a devastating parasitic disease in many tropical and subtropical countries and accounts for about 2 million deaths every year. (Sudhanshu *et al*., 2003). Most cases of malaria and deaths are caused by *Plasmodium falciparum* which develops in the gut of the mosquito and

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is passed on in the saliva of an infected insect each time it takes a new blood meal and infected mosquito bites a human, it rapidly reaches the liver within 30 minutes and reproduces hastily and enter RBC, spread in the host's blood (Nutan Prakash *et al.*, 2010). The disease primarily affects poor populations in tropical and subtropical areas, where the temperature and rainfall are suitable for the development of vectors and parasites (Snow *et al.*, 2005). *P. falciparum* infection can have serious effects, for example, anemia, cerebral complications (from coma to convulsions), hypoglycemia and glomerulonephritis. The disease is most serious in the non-immune individuals, including children; pregnant women and tourists (Kirandeep Kaur *et al.*, 2009).The development of resistance to mainstay drugs like chloroquine, and controlled use of new artemisinin analogs have created an urgent need to discover new antimalarial agents.

Many anti-malarial drugs are used to treat malaria one of the most important drug is Proguanil which is a prophylactic antimalarial drug, which works by stopping the malaria parasite, *Plasmodium falciparum* and *Plasmodium vivax*, from reproducing once it is in the red blood cells. It does this by inhibiting the enzyme, dihydrofolate reductase, which is involved in the reproduction of the parasite. Dihydrofolate reductase is a small enzyme which manages the state of folate, a snaky organic molecule that shuttles carbon atoms to enzymes that need them in their reactions of particular importance, the enzyme thymidylate synthase uses these carbon atoms to build thymine bases, an essential component of DNA. After the release of folate carbon atoms has been recycled by dihydrofolate reductase. Sometimes, overdose of Proguanil also creates problems. Dihydrofolate reductase has been a primary target for the Proguanil drug (Nutan Prakash *et al.*, 2010).

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor (Richon, 1994). Ligand binding interactions are central to

numerous biological process. As many proteins regulate key biological functions via interactions with small molecules, these receptor proteins are often prime targets for therapeutic agents.

Nature remains an ever evolving source for compounds of medicinal importance. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times. For example, use of *Cinchona succiruba* (Rubiaceae) for the treatment of malaria infection is known for centuries. Several compounds isolated from nature also form a rich source of diverse structures for optimization to obtain improved therapeutics (Kumar *et al*., 2002). Hence for this present study Chromolaena odorata plant was selected which belongs to the family Asteraceae and has been classified as weed. It is a native of Central and South America which has spread throughout the tropical and subtropical areas of the world. It is a perennial, diffuse and scrambling shrub which grows to 3-7m in height when growing in the open. It is now a major weed that is widespread in central and western Africa, tropical America, West India and Southeast Asia and western part in Nigeria (Ling *et al*., 2007). There are more than 20 bio active compounds has been derived from this plant through GC MS analysis. The aim of the present study is to investigate the inhibitory activity of the derived compounds on malarial receptor and an understanding of drug–receptor interactions, which enables a modification of the drug's structure to achieve suitable interactions by molecular docking studies. We also find the suitable analogues with high binding affinity, which could be a possible lead molecule by using the Accelrys – Discovery Studio 2.1software.Hence it would serve as a development of new and more effective drugs for malaria.

2. MATERIALS AND METHODS

2.1 Plant material collection

The fresh leaves of *C. odorata* were collected from the Mother Teresa Women's University campus, Kodaikanal, Dindugal district, Tamil Nadu, India. It was authenticated by a taxonomist Dr.P.Jeyaraman, National Institute of Herbal Science, PARC, Chennai. Voucher specimen has been deposited in the University laboratory of Biotechnology. The collected leaves

were washed thoroughly with distilled water for removing contaminants. The thoroughly washed plant materials were dried in shade and the dried leaves were powdered using an electrical blender. The powdered plant materials were stored in airtight, dark, glass container and refrigerated for further analysis.

2.2 Extraction and GC-MS Analysis:

Ten grams of powdered samples of *C. odorata* were successively extracted with 50mL of methanol using Soxhlet apparatus. The extracts were filtered through a filter paper and finally dried in rotary evaporator. After removal of the methanol in a rotary evaporator at a temperature below 40 ◦C these extracts were used for GC-MS analysis.

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ionsource temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass. Interpretation on mass spectrum GC-MS was conducted using the database of national Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. (Sutha *et al*., 2011)

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2.3 Protein Data Bank

The Protein Data Bank (PDB) was a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The 3-D structure of the anti-malarial receptor DHFR was retrieved from the protein data bank (PDB) (WWW.rcsb.org/pdb). PDB (ID: 2FK4). Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and pymol molecular visualization software.

2.4 Lipinski's Analysis

Lipinski's rule says that to evaluate drug likeness and determine the pharmacological activity. The Lipinski's properties like molecular weight, log p, number of hydrogen bond donors and acceptors. Lipinski's parameters to satisfy the retrieved phytocompounds of *Chromolaena odorata* were analyzed, using PubChem tool **(**Lipinski C A., 2000**).**

2.5 PubChem Compound:

PubChem is a database of chemical molecules. PubChem contain substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to growing PubChem database. PubChem Compound was a searchable database of chemical structures with validated chemical depiction information provided to describe substances in PubChem Substance. Structures stored within PubChem Compounds are pre-clustered and cross-referenced by identify and similarity groups.

2.6 Chemsketch

Chemsketch is designed to be used on its own for drawing chemical structures, reactions, schematic diagrams or integrated with other ACD applications and as the front end to our software. Able to import Windows Metafile, MDL MOL, CS ChemDraw, or ISIS/Sketch BIN file. Export Bitmap, TIFF, Metafile, MOL, Paintbrush, ISIS/Sketch, GIF, and ChemDraw. Fully loaded with useful pre-drawn structures including lab equipment, DNA/RNA building kit, amino acids etc. Structures can be 2D "cleaned" as well as 3D optimized using ACD's powerful

algorithm. Publish a professional quality report from within ChemSketch or drag drop structures/text into MS applications.

2.7 Accelrys – Discovery Studio 2.1

Accelrys had provided software for chemical research, especially in the areas of drug discovery and material science. Accelrys manages a Nanotechnology Consortium producing software tools for rational nano design. The determination of the ligand binding affinity was calculated using LigScore and PLP1, JAIN and Dock score were used to estimate the ligand-binding energies. Apart from these, other input parameters for docking were set as default options. Thus docking analysis of ligand molecules from *Chromolaena odorata* with Dihydrofolate reductase was carried out by Ligand Fit of Discovery studio (Version 2.1, Accelry's Software Inc.). The software allows us to virtually screen a database of compounds and predicts the strongest binders based on various scoring functions. A molecular mechanics like scoring function and number of hydrogen bonds is employed by dock score to rank the docked possess **(**Daisy P *et al*., 2011**).** It explores the ways in which those ligand molecules and the enzyme Dihydrofolate reductase fit together and dock to each other well, like pieces of a three-dimensional jigsaw puzzle. The collection of ligand molecules and Dihydrofolate reductase complexes was identified via docking and their relative stabilities were evaluated using their binding affinities.

Results and Discussion:

Chromolaena odorata Linn. Was commonly called as Siam weed plant traditionally used in wound dressing. (Afolabi *et al*., 2007). The leaves of *Chromolaena odorata* has been reported to contain alkaloids, saponins, tannins, flavonols, flavonones, chalcones and phenolic acids (Phan *et al*., 2001). Quercetin and kaempferol have been known to be potent anti-oxidant, anti-inflammatory and anti-diabetic agents. (Onkaramurthy *et al.,* 2013). *Chromolaena odorata* Linn., is traditionally used to manage diabetes (Balasubashini *et al.*, 2004; Harini and Pugalendi, 2010) and in eye problems

(Duke *et al*.,1929; Pullaiah and Naidu, 2003). It also showed insecticidal properties against the dengue vector *Aedes aegypti* (Rajmohan D and Logan Kumar K., 2011)

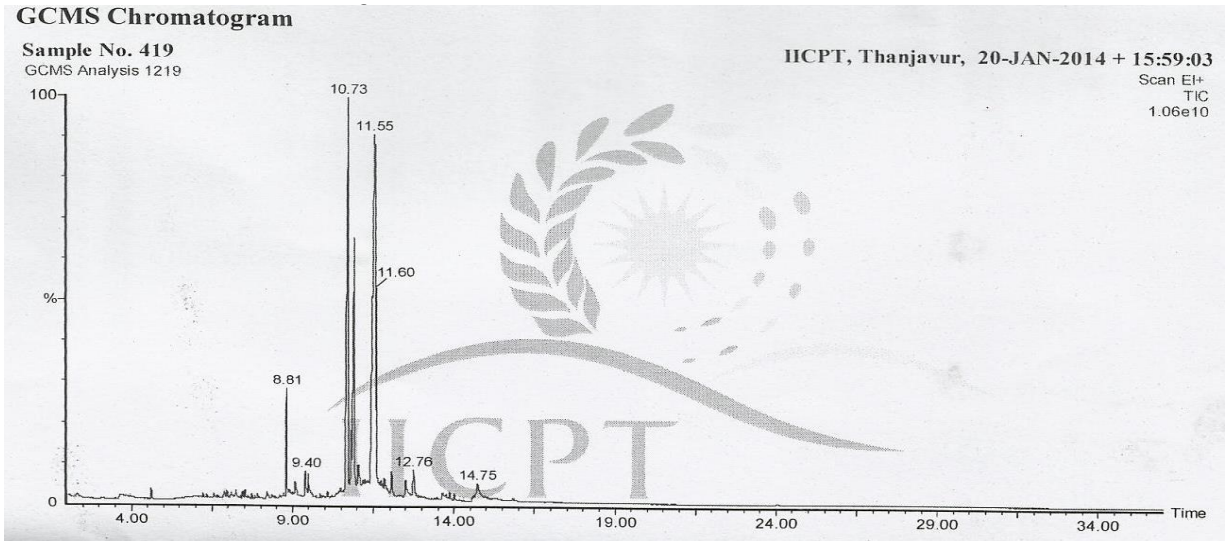
In the present study Methanolic extract of the leaves of *Chromolaena odorata* was subjected to GC-MS analysis to identify the potential phytocompounds through which drug has been found by Molecular docking. There are twenty chemical constituents have been identified from Methanolic extract of the whole plant of *Vernonia cinerea* (P. Abirami and A. Rajendran , 2012) and *Eupatorium triplinerve* (G Selvamangai and Anusha Bhaskar, 2012) showed ten compounds by GC-MS analysis. In our study the GC-MS analysis clearly showed the presence of 33 compounds in (table 1) and the chromatogram is shown in (Fig 1).Out of these compounds 4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone showed maximum peak area of 38.09%, followed by 2H-Inden-2-one,1,4,5,6,7,7a-hexohydro-4-methyl-7-(1-methylethyl)-l and 9,12-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-with 21.20% and 5.55% respectively. All these phytocompouds and the receptor DHFR were subjected to predict the antimalarial potential through *in silico* docking analysis by using the commercial docking software Accelrys – Discovery Studio 2.1. DHFR receptor is considered to play an important role in inhibiting the malarial parasitic activities and found as the most active compound in the respective target site.

Table 1 Phyto-components identified in the *Chromolaena odorata* through GC MS

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	4.62	Borynyl acetate	$C_{12}H_{20}O_2$	196	0.93
2	6.24	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	$C_{15}H_{24}$	204	0.46
3	6. 34	Trans- à –Bergamotene	$C_{15}H_{24}$	204	0.72
4	6.56	1-(Cyclopropyl-nitro-methyl)-cyclopentanol	$C_9H_{15}NO_3$	185	0.58

5	6.88	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethynyl)-, [1R-(1à,3a á,4 à,7á)]	$C_{15}H_{24}$	204	0.69
6	6.97	Bergamotol,Z- à -trans-	$C_{15}H_{24}O$	220	0.71
7	7.10	Z,Z,Z-4,6,9-Nonadecatreine	$C_{19}H_{34}$	262	0.76
8	7.24	Cyclohexane, 1-methyl1-4-(5-methyl-1-methylene-4-hexenyl)-, (S)	$C_{15}H_{24}$	204	0.70
9	7.46	Cedrene	$C_{15}H_{24}$	204	0.33
10	7.53	á–Vatirenene	$C_{15}H_{22}$	202	0.39
11	7.74	Ledene oxide-(II)	$C_{15}H_{24}O$	220	0.26
12	7.92	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-,[S-()Z]-	$C_{15}H_{26}O$	222	0.37
13	8.20	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1aà,4aà,7 á,7a á,7bà)]-	$C_{15}H_{24}O$	220	0.39
14	8.37	12,15-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.24
15	8.72	Cyclohexanemethanol, 4-ethenyl-a,a,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4 á)]-	$C_{15}H_{26}O$	222	0.16
16	8.81	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1S-(1aà,4à,4aà,8aà,)]-	$C_{15}H_{26}O$	222	4.69
17	9.10	2-Naphthalenemethanol, decahydro-à, à,4a-trimethyl-8-methylene-,[2R-(2à,4a á,8a á)]-	$C_{15}H_{26}O$	222	1.17
18	9.40	à-Bisabolol	$C_{15}H_{26}O$	222	1.94
19	9.49	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	$C_{15}H_{22}O$	218	2.20
20	10.73	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	$C_{15}H_{22}O_2$	234	38.09
21	10.82	Cis-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220	2.23
22	10.92	2H-Inden-2-one,1,4,5,6,7,7a-hexohydro-4-methyl-7-(1-methylethyl)-	$C_{13}H_{20}O$	192	21.20
23	11.55	Bicyclo [5.1.0]octan-2-one, 4,6-diisoprppylidene-8,8-dimethyl-	$C_{16}H_{24}O$	232	5.33
24	11.60	Longipinocarvone	$C_{15}H_{22}O$	218	1.27

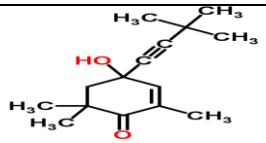
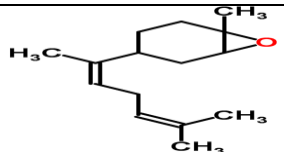
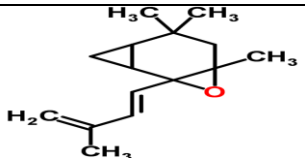
25	12.06	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8,-tetramethyl-,[3R-(3à,3a ά,7 ά,8aà)]-	$C_{15}H_{24}$	204	1.46
26	12.51	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.99
27	12.76	Falcarinol	$C_{17}H_{24}O$	244	2.25
28	13.65	1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-	$C_{16}H_{22}O$	230	0.87
29	13.86	9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.44
30	14.02	Phytol	$C_{20}H_{40}O$	296	0.40
31	14.61	9,12-Octadecatrienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.61
32	14.75	9,12-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	5.55
33	15.84	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	0.60



The goal of ligand-protein docking is to predict the predominant binding model of a ligand with a protein of known three dimensional structure. (Mittal *et al*., 2009) .To study the binding modes of bioactive compounds in the binding site of DHFR receptor**,** intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations

of the receptor ligand complexes.(Srivastava *et al*., 2010). Lipinski's rule five is important for drug development where a pharmacologically active phytocompounds should have not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, Partition coefficient A Log P less than 5. (Lipinsky *et al.*, 2000) Out of the thirty three identified phytocompouds four compounds satisfied Lipinski properties showed positive interaction in docking and the results are presented in [Table-2].

Table 2 Lipinski's properties of the four compounds of *Chromolaena odorata*

Ligand molecule	**Molecular weight [g/mol]**	**Molecular Formula**	**Xlogp3 value (<=5)**	**H bond Donor**	**H-bond acceptor**	**Structure**
4-(3,3-dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	234	$C_{15}H_{22}O_2$	2.96	1	2	
4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	218	$C_{15}H_{22}O$	3.931	0	1	
Cis-Z-a-Bisabolene epoxide	220	$C_{15}H_{24}O$	4.3	0	1	

Falcarinol	244	$C_{17}H_{24}O$	5.5	1	1	

The phytocompounds namely 4-(3,3-dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone, 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane, Cis-Z-a-Bisabolene epoxide and Falcarinol satisfied the Lipinski's properties (Table-2).The identified phytocompouds exhibited the docking energy between 13.398 to - 71.128 Kcal/mol. Of which, the maximum docking energy was found in Falcarinol (-71.128 Kcal/mol). Higher docking energy shows good binding energy and hence more efficient in blocking the activity of the particular protein. A significant amount of stability is conferred upon the drug-receptor interaction when multiple hydrogen bonds, high dock score value and low bond length are formed between drugs and receptor. (Trapani *et al*., 1992). Hence the present study, the interaction between Falcarinol and DHFR receptor conferred a significant amount of stability when compared to other compounds (Table-3). Falcarinol showed high dock score value i.e. 71.128 Kcal/mol and it produced strong hydrogen bond interactions with the residues GLU 321 (1 hydrogen bond) of the receptor whereas other compounds produced low dock score value which will minimize the interaction between ligand and the receptor. Analysis of ligand binding interaction with the DHFR receptor can be useful for new preventive and therapeutic drug for malaria. The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores.

Figure 2-6: Interaction of drug-receptor complexes

Fig 2: 4-(3, 3-dimethyl-but-1-ynyl)-4-hydroxy-2, 6, 6-trimethylcyclohex-2-enone

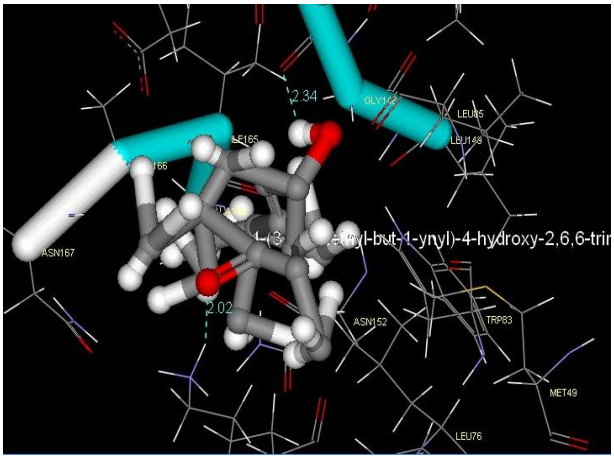


Fig 3: 4, 6, 6-trimethyl-2-(3-methylbuta-1, 3-dienyl)-3-oxatricyclo [5.1.0.0(2, 4)] octane

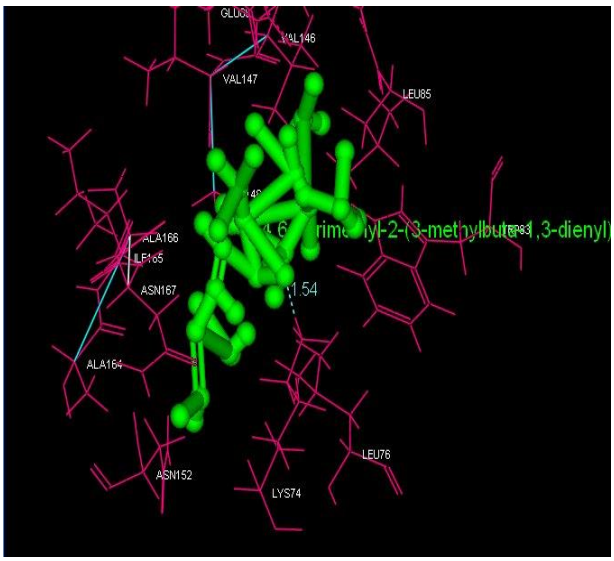


Fig 4: Cis-Z-a-Bisabolene epoxide

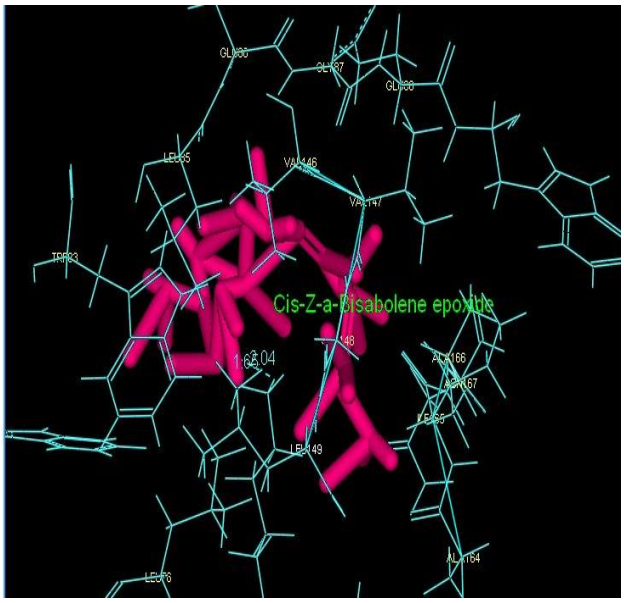


Fig 5: Falcarinol

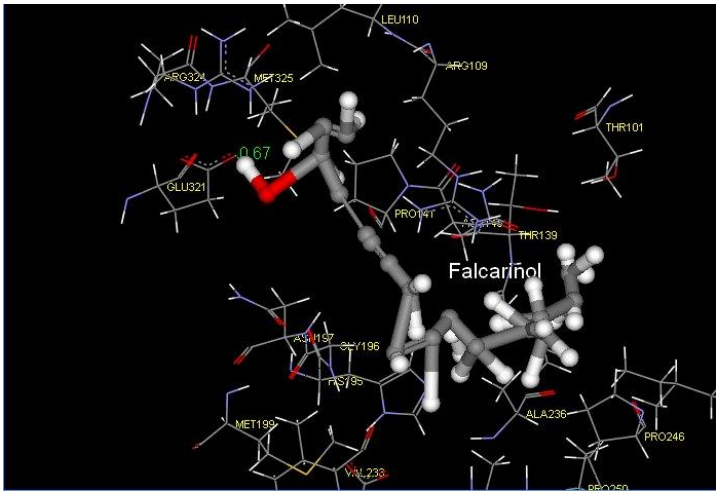


Table: 3: Molecular Docking analysis for the identification of bioactive compounds against Malaria

Ligand molecule	Amino acid	Atoms in amino acid	Position	Atoms in ligand	H-bond length **(Å)**	Dock score
4-(3,3-dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	LYS VAL	74 174	HZ2 H30	O2 O1	2.34 2.02	13.398
4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)] octane	LYS	74	HZ2	O1	1.54	31.18
Cis-Z-a-Bisabolene epoxide	LYS LYS	74 74	HZ2 HZ3	O1 O1	2.04 1.65	29.484
Falcarinol	GLU	321	H40	OE2	0.67	71.128

CONCLUSION

The Protein-Ligand interaction plays a vital role in structural based drug designing. In the present work there are thirty three phytocompounds have been identified from Methanolic extract of the leaves of *Chromolaena odorata* by Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive Compounds justifies the use of the leaves of the plant various ailments by traditional practitioners. In this study we have docked the receptor dihydrofolate reductase with thirty three phytocompounds. Out of 4 compounds the only one

bioactive compounds of *C.odorata* i.e. falcarinol holds more promising drug formation against malaria based on docking analysis (minimum hydrogen bond length and maximum dock score). Further *in-vivo* and *in-vitro* approaches are required to elucidate the molecular mechanisms of this compound to act as potent drug against malaria.

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